OPEN

Positivity and Risk Factors for *Trichomonas vaginalis* Among Women Attending a Sexual Health Clinic in Melbourne, 2006 to 2019

Esha Abraham, BMedSc(Hons),† Christopher K. Fairley, PhD,*† Ian Denham, MBBS,* Catriona S. Bradshaw, PhD,*†‡ Rebecca M. Farquharson, BMedSc(Hons),*† Lenka A. Vodstrcil, PhD,*†‡ Erica L. Plummer, PhD,*† Jason J. Ong, PhD,*† Marcus Y. Chen, PhD,*† Tiffany R. Phillips, PhD,*† and Eric P.F. Chow, PhD*†‡*

Background: *Trichomonas vaginalis* is not a notifiable disease in Australia in most states, resulting in limited Australian epidemiological studies. This study aimed to examine the positivity of *T. vaginalis* in women attending the Melbourne Sexual Health Centre (MSHC) and identify associated factors.

Methods: All women 16 years or older who were tested for *T. vaginalis* at MSHC from 2006 to 2019 were included. The diagnostic method changed from culture to nucleic acid amplification test in August 2018. The annual positivity of *T. vaginalis* was calculated. Because of the data completeness, we performed a generalized estimating equations multivariable logistic regression using data from 2011 to 2019 to examine factors associated with *T. vaginalis* positivity.

Results: From 2006 to 2019, 69,739 tests for *T. vaginalis* were conducted, and 294 tested positive (0.42%; 95% confidence interval [CI], 0.37%–0.47%). Approximately 60% of women tested reported symptoms. After adjusting for potential confounders including the change in diagnostic method, there was a 21% (95% CI, 12%–31%) annual increase in *T. vaginalis* positivity between 2011 and 2019. Women with concurrent syphilis had the highest odds of testing positive for *T. vaginalis* (adjusted odds ratio [aOR], 21.55; 95% CI, 6.96–66.78), followed by women who had injected drugs in the last 12 months (aOR, 6.99; 95% CI, 4.11–11.87), were 35 years or older (aOR, 3.47; 95% CI, 2.26–5.35), or had concurrent chlamydia (aOR, 1.77; 95% CI, 1.05–2.99).

Conclusions: The rising positivity of *T. vaginalis* at MSHC irrespective of change in diagnostic method suggests a concurrent community-wide rise in Melbourne. Given the rising positivity, testing informed by risk factors should be considered.

With an estimated incidence of 156 million cases each year,[1] *Trichomonas vaginalis* is one of the most common curable, nonviral sexually transmitted infections (STIs) worldwide.[2] Although initially viewed as a nuisance because of its oft-asymptomatic nature,[3] current research has revealed its role in reproductive complications,[4,5] HIV acquisition,[6–9] and cervical cancer.[10]

In Australia, *T. vaginalis* is not a notifiable condition except in the Northern Territory, and therefore, there are no national surveillance data available. Most epidemiological studies have estimated the test positivity of *T. vaginalis* to be less than 1% in women living in urban Australian cities. However, women in rural and remote areas are disproportionately impacted; a 2015 study conducted in 68 remote Aboriginal communities reported a *T. vaginalis* positivity of 24% among women.[11] A study focusing on women presenting to public sexual health clinics in rural and remote areas of western New South Wales found a positivity of 8% (95% confidence interval [CI], 6%–12%). Outside of major cities, access to healthcare is much more limited because it takes longer and is more costly to access services, both of which impact an individual's decision to present for testing.[12] Furthermore, asymptomatic individuals are simply less likely to present for STI screening.[13,14] Other risk factors for trichomoniasis identified in previous literature include older age,[15,16] use of injecting drugs,[16] and Indigenous status.[16] Research has shown an increase in the prevalence of other STIs, such as gonorrhea and syphilis, in heterosexuals in Australia.[17,18] Given the paucity of data on *T. vaginalis*, it is important to determine whether there have been increases in *T. vaginalis* positivity as well.

From the *Melbourne Sexual Health Centre, Alfred Health; †Central Clinical School, Faculty of Medicine, Nursing and Health Sciences, Monash University; and ‡Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Victoria, Australia

T.R.P. and E.P.F.C. are co-last authors.

Acknowledgments: The authors thank Afrizal Afrizal at the Melbourne Sexual Health Centre for his assistance with data extraction at the Melbourne Sexual Health Centre.

Conflict of Interest and Sources of Funding: The authors have no conflicts of interest to declare. E.P.F.C. is supported by an Australian National Health and Medical Research Council Emerging Leadership Investigator Grant (GNT1172873). C.K.F. and C.S.B. are each supported by an Australian National Health and Medical Research Council Leadership Investigator Grant (GNT1172900 and GNT1173361, respectively).

Author Contributions: E.P.F.C. and C.K.F. conceived and designed the study. E.A. performed literature review, conducted data analysis, and wrote the first draft of the manuscript. T.R.P. and E.P.F.C. provided supervision of the project and statistical advice. E.A., C.K.F., I.D., C.S.B., R.M.F., L.A.V., E.L.P., J.J.O., M.Y.C., T.R.P., and E.P.F.C. were involved in data interpretation, revising the manuscript for important intellectual content and approved the final version.

Correspondence: Esha Abraham, BMedSc(Hons), Melbourne Sexual Health Centre, 580 Swanston St, Carlton, VIC 3053, Australia. E-mail: abraham.esha@gmail.com; Eric P.F. Chow, PhD, Melbourne Sexual Health Centre, 580 Swanston St, Carlton, VIC 3053, Australia. E-mail: eric.chow@monash.edu.

Received for publication May 8, 2022, and accepted July 29, 2022.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (http://www.stdjournal.com).

DOI: 10.1097/OLQ.0000000000001690

This study aimed to examine the trends in *T. vaginalis* positivity among women and to identify the factors associated with *T. vaginalis* positivity among women attending an urban sexual health clinic in Melbourne, Australia.

METHODS

Study Setting and Population

This was a retrospective repeated cross-sectional study analyzing data collected from electronic medical records collected at the Melbourne Sexual Health Centre (MSHC) between January 1, 2006, and December 31, 2019. The MSHC is the largest public sexual health clinic in Australia, providing more than 50,000 consultations in 2019. During the study period, the clinic operated as a walk-in service with no referral required for consults.

Upon arrival, clients completed a questionnaire through a computer-assisted self-interview (CASI), which collected demographic characteristics (e.g., age, country of birth, number of years in Australia, and Indigenous status), sexual practices over the last 12 months (e.g., number of partners, condom use, and sex overseas or with someone from overseas), and current sex worker status. Clients were categorized as "Unknown" or "Declined to answer" if they chose not to answer a question.

Among women who were born overseas, the median time between when they arrived in Australia and the date that they attended MSHC was 2 years. Thus, clients were categorized into "Australian, or New Zealand born, or overseas-born who resided in Australia >2 years" or "overseas-born who resided in Australia ≤2 years." Residential postcode was used to determine the location of residence per the Australian Bureau of Statistics Remoteness Structure classification.[19]

In this study, we included all women 16 years or older who attended the MSHC and were tested for *T. vaginalis* between 2006 and 2019. Women with symptoms such as abnormal vaginal discharge were tested for *T. vaginalis*. In addition, some asymptomatic women were also tested for *T. vaginalis* based on the individual's risk profile (e.g., women living with HIV, female sex workers, had sexual contact with a partner with *T. vaginalis*) and clinical decision. The guidelines for testing for *T. vaginalis* did not change at the MSHC over the study period, and screening of asymptomatic women without risk factors was not recommended.

We excluded transgender individuals because CASI did not differentiate between transmen and transwomen. Any indeterminate or invalid nucleic acid amplification test (NAAT) results for *T. vaginalis* were excluded because they were followed up with a repeat test that was then included in the analysis.

Laboratory Methods

All endocervical specimens included in the study were collected by clinicians. During the study period, the MSHC used 2 diagnostic methods for *T. vaginalis*. Before August 2018, culture was used to diagnose *T. vaginalis* at the MSHC. Samples were placed in Diamond's medium and incubated at 37°C for a minimum of 48 hours before inspecting for trichomonads. In August 2018, the MSHC changed the diagnostic method for *T. vaginalis* from culture to NAAT using the Aptima Combo-2 (AC2) assay (Hologic Gen-Probe Panther system; Hologic, San Diego, CA). Wet prep microscopy was occasionally ordered if there was a clinical suspicion of *T. vaginalis*; however, these results were excluded because culture or NAAT was used to confirm the findings.

There were changes in the diagnostic method used for other infections. Before March 2015, *Chlamydia trachomatis* was diagnosed by NAAT BD ProbeTec Strand Displacement Amplification Assay (Becton Dickinson, Sparks, MD) amplified DNA Assays, and this was replaced by the AC2 assay from March 2015 onward. In March 2015, gonorrhea diagnosis was changed from culture to NAAT using the AC2 assay. Syphilis was serologically diagnosed by enzyme immunoassay, *Treponema pallidum* particle agglutination assay, and the rapid plasma reagin test. *T. pallidum* polymerase chain reaction was used on suspected primary syphilis lesions. Positive laboratory results were then correlated with client history by an experienced sexual health physician to confirm staging. Our analyses included primary, secondary, and early latent syphilis. Bacterial vaginosis (BV) was recorded as a clinical diagnosis during consultations, and thus, the diagnosis was categorized into "Yes" or "No." However, BV is diagnosed routinely at the MSHC using both Amsel's and Nugent score (BV defined as ≥3 Amsel criteria and Nugent score of 4–10). Women were classified as symptomatic if they reported symptoms to the triage nurse, and asymptomatic if they reported no symptoms.

Statistical Analysis

The test positivity of *T. vaginalis* was calculated as the number of women who tested positive divided by the number of women tested and stratified by calendar year. The 95% CIs of the positivity were calculated using the binomial exact method.[20] The χ^2 trend test was used to examine the annual trend of positivity. The positivity of *T. vaginalis* was also stratified by the diagnostic method, and a χ^2 test was used to examine the difference between the diagnostic methods (i.e., culture vs. NAAT).

Logistic regression models with generalized estimating equations (GEEs) were built to examine the risk factors associated with *T. vaginalis* positivity. However, we only included data between March 2011 and December 2019 in this analysis because syphilis testing data were not available in the electronic medical records before March 2011. We considered it important to include syphilis as a risk factor in the analysis because there has been a rise in syphilis cases among women during this period in Victoria, Australia.[17,21] Study variables with a P value <0.10 in the univariable logistic GEE models were considered as potential confounding factors and were included in the multivariable logistic GEE model. The GEE model with an exchangeable correlation structure was used to account for multiple visits or testings from the same individual over the study period.

All analyses were performed using Stata (Version 14; StataCorp LP, College Station, TX). Ethics approval was granted by the Alfred Hospital Ethics Committee, Melbourne, Australia (210/20).

RESULTS

There were 69,739 tests for *T. vaginalis* performed among 28,760 individuals between 2006 and 2019. The median age was 29 years (interquartile range [IQR], 25–36 years). Most women were born in Australia or New Zealand or had lived in Australia for more than 2 years (59%; n = 19,866). Table 1 provides a summary of the basic demographic information.

Test Positivity of *T. vaginalis*

There were 294 *T. vaginalis* cases diagnosed among 251 individuals during the study period. The overall test positivity of *T. vaginalis* between 2006 and 2019 was 0.42% (294 of 69,739; 95% CI, 0.37%–0.47%). The test positivity increased significantly from 0.27% (95% CI, 0.15%–0.45%) in 2006 to 1.57% (95% CI, 1.17%–2.06%) in 2019 ($P_{trend} < 0.001$). Figure 1 demonstrates the number of *T. vaginalis* cases and test positivity by year throughout the study period. Supplementary Table 1, http://links.lww.com/OLQ/A848, provides a breakdown of *T. vaginalis* test results by year.

Culture was used to diagnose patients from January 2006 to July 2018, when it was replaced with NAAT. The positivity by

TABLE 1. Factors Associated With *T. vaginalis* Test Positivity Among Women Attending the Melbourne Sexual Health Centre, 2011 to 2019

Characteristic	Tests, %	Test Positive, %*	OR (95% CI)	*P*	aOR (95% CI)	*P*
Year	33,895 (100.00)	205 (0.60)	1.27 (1.20–1.34)	<0.001	1.21 (1.12–1.31)	<0.001
Method of testing†						
Culture	29,548 (87.18)	132 (0.45)	1	Reference	1	Reference
NAAT	4347 (12.82)	73 (1.68)	3.48 (2.56–4.75)	<0.001	1.47 (0.95–2.28)	0.087
Age group, y						
16–24	9229 (27.23)	37 (0.40)	1	Reference	1	Reference
25–34	17,032 (50.25)	82 (0.48)	1.14 (0.76–1.72)	0.530	1.27 (0.84–1.91)	0.250
≥35	7634 (22.52)	86 (1.13)	3.14 (2.09–4.72)	<0.001	3.47 (2.26–5.35)	<0.001
Indigenous status						
No	26,647 (78.62)	157 (0.59)	1	Reference	1	Reference
Yes	270 (0.80)	6 (2.22)	3.26 (1.25–8.48)	0.016	1.66 (0.61–4.52)	0.318
Unknown or declined to answer	6978 (20.59)	42 (0.60)	1.13 (0.79–1.63)	0.503	1.00 (0.68–1.47)	0.989
Location of residence‡						
Urban	24,725 (72.95)	160 (0.65)	1	Reference		
Regional, rural, or remote	855 (2.52)	6 (0.70)	1.27 (0.55–2.95)	0.571		
Interstate	495 (1.46)	0 (0.0)	—	—		
Unknown or declined to answer	7820 (23.07)	39 (0.60)	0.80 (0.55–1.17)	0.257		
Time in Australia						
Born in Australia or New Zealand or >2 y	19,866 (58.61)	129 (0.65)	1	Reference	1	Reference
≤2 y	10,257 (30.26)	46 (0.45)	0.66 (0.46–0.94)	0.020	0.80 (0.55–1.16)	0.242
Unknown or declined to answer	3772 (11.13)	30 (0.80)	1.29 (0.84–1.97)	0.249	1.32 (0.84–2.05)	0.227
Injecting drug use in the last 12 mo						
No	25,193 (74.33)	125 (0.50)	1	Reference	1	Reference
Yes	463 (1.37)	24 (5.15)	8.14 (4.93–13.44)	<0.001	6.99 (4.11–11.87)	<0.001
Unknown or declined to answer	8239 (24.31)	56 (0.68)	1.40 (1.03–1.91)	0.031	0.67 (0.35–1.29)	0.227
No. male partners in the last 12 mo						
≤1	16,128 (47.58)	65 (0.61)	1	Reference		
>1	13,517 (39.88)	79 (0.52)	0.78 (0.56–1.08)	0.133		
Unknown or declined to answer	4250 (12.54)	61 (0.76)	1.24 (0.89–1.73)	0.199		
Condomless sex with male partners in the last 12 mo						
No or no sex	4088 (12.06)	14 (0.34)	1	Reference	1	Reference
Yes	17,298 (51.03)	113 (0.65)	1.77 (1.03–3.04)	0.038	1.54 (0.86–2.74)	0.143
Unknown or declined to answer	12,509 (36.91)	72 (0.62)	1.87 (1.08–3.23)	0.026	1.24 (0.64–2.42)	0.520
Gender of sexual partners in the last 12 mo						
Male only	20,043 (59.13)	120 (0.60)	1	Reference		
Male and female	2231 (6.58)	15 (0.67)	1.12 (0.65–1.92)	0.691		
Female only	412 (1.22)	2 (0.49)	0.92 (0.24–3.51)	0.904		
Unknown or declined to answer	11,209 (33.07)	68 (0.61)	1.13 (0.84–1.52)	0.424		
Current sex worker						
No	10,616 (31.32)	102 (0.63)	1	Reference	1	Reference
Yes	15,203 (44.85)	54 (0.40)	0.75 (0.53–1.06)	0.109	0.78 (0.52–1.20)	0.260
Unknown or declined to answer	8076 (23.83)	49 (1.15)	1.73 (1.22–2.47)	0.002	2.02 (1.03–3.97)	0.041
Sex overseas or with someone from overseas in the last 12 mo						
No	15,711 (46.35)	95 (0.60)	1	Reference		
Yes	9173 (27.06)	52 (0.57)	0.89 (0.64–1.25)	0.507		
Unknown or declined to answer	9011 (26.59)	58 (0.64)	1.07 (0.78–1.47)	0.692		
Self-reported previous STI diagnosis						
No	13,631 (40.22)	60 (0.44)	1	Reference	1	Reference
Yes	7037 (20.76)	45 (0.64)	1.30 (0.88–1.92)	0.181	1.03 (0.69–1.54)	0.894
Unknown or declined to answer	13,227 (39.02)	100 (0.76)	1.71 (1.25–2.34)	0.001	1.54 (1.03–2.32)	0.036
Concurrent *C. trachomatis*						
No	30,748 (90.72)	170 (0.55)	1	Reference	1	Reference
Yes	1700 (5.02)	17 (1.00)	1.66 (1.00–2.75)	0.051	1.77 (1.05–2.99)	0.033
Not tested	1447 (4.27)	18 (1.24)	1.71 (1.00–2.95)	0.052	1.53 (0.62–3.76)	0.353
Concurrent *N. gonorrhoeae*						
No	32,224 (95.07)	180 (0.56)	1	Reference	1	Reference
Yes	261 (0.77)	8 (3.07)	4.62 (2.16–9.89)	<0.001	2.26 (1.00–5.14)	0.051
Not tested	1410 (4.16)	17 (1.21)	1.62 (0.93–2.83)	0.091	1.16 (0.46–2.89)	0.751
Concurrent syphilis						
No	18,653 (55.03)	116 (0.62)	1	Reference	1	Reference
Yes	22 (0.06)	6 (27.27)	57.61 (22.23–149.34)	<0.001	21.55 (6.96–66.78)	<0.001
Not tested	15,220 (44.90)	83 (0.55)	0.88 (0.67–1.16)	0.359	0.93 (0.68–1.27)	0.667

Continued next page

TABLE 1. *(Continued)*

Characteristic	Tests, %	Test Positive, %*	OR (95% CI)	*P*	aOR (95% CI)	*P*
HIV status						
Negative	31,317 (92.39)	191 (0.61)	1	Reference		
Positive	282 (0.83)	3 (1.06)	1.83 (0.59–5.66)	0.296		
Unknown or declined to answer	2296 (6.77)	11 (0.48)	0.79 (0.44–1.44)	0.445		
Concurrent bacterial vaginosis						
No	28,731 (84.76)	175 (0.61)	1	Reference		
Yes	5164 (15.24)	30 (0.58)	0.87 (0.59–1.29)	0.492		

*The percent refers to the proportion of positive tests from a subgroup (e.g., culture or NAAT).

†Culture was the primary diagnostic method from January 2006 to July 2018. NAAT was the primary diagnostic method from August 2018 to December 2019.

‡Residential postcode was stratified using data from the Australian Bureau of Statistics Remoteness Structure classification.

aOR indicates adjusted odds ratio; CI, confidence interval; HIV, human immunodeficiency virus; NAAT, nucleic acid amplification test; OR, odds ratio; STI, sexually transmitted infection.

culture from January 2006 to July 2018 (0.34%; 95% CI, 0.30%–0.39) was lower than the positivity recorded by NAAT from August 2018 to December 2019 (1.68%; 95% CI, 1.32%–2.11%). From January to July 2018, the positivity of TV by culture was 1.44% (25 of 1732). In comparison, the positivity by NAAT from August to December 2018 was 1.99% (23 of 1157). The test positivity of *T. vaginalis* also increased significantly from 0.27% (14 of 5238) to 1.44% (25 of 1732) during the culture-only period ($P_{trend} < 0.001$); however, the test positivity of *T. vaginalis* by NAAT did not change significantly from August 2018 to December 2019.

The median age of women who tested negative for *T. vaginalis* (29 years; IQR, 25–39 years) was significantly lower than women who tested positive for *T. vaginalis* (32 years; IQR, 26–42 years; $P < 0.001$). Approximately 60% of women tested reported symptoms when they presented to the clinic (58.07% [40,499 of 69,739]). The positivity of *T. vaginalis* among symptomatic women (0.62%; 95% CI, 0.52%–0.71%; 180 of 29,240) was significantly higher than asymptomatic women (0.28%; 95% CI, 0.23–0.34; 114 of 40,499; $P < 0.001$).

Factors Associated With *T. vaginalis*

There was a 21% (95% CI, 12%–31%) annual increase in *T. vaginalis* positivity between 2011 and 2019 after adjusting for method of testing, age group, Indigenous status, time in Australia, injecting drug use in the last 12 months, condomless sex with male partners in the last 12 months, sex worker status, previous STI diagnosis, concurrent *C. trachomatis,* concurrent *Neisseria gonorrhoeae,* and concurrent syphilis (Table 1).

After adjusting for other factors, women with concurrent syphilis had the highest odds of testing positive for *T. vaginalis* compared with women who did not test positive for syphilis (adjusted odds ratio [aOR], 21.55; 95% CI, 6.96–66.78; $P < 0.001$). Women who had injected drugs in the last 12 months were at a higher risk of testing positive in comparison to women who had not injected drugs in the last 12 months (aOR, 6.99; 95% CI, 4.11–11.87; $P < 0.001$). Women 35 years or older were more likely to test positive than women aged 16 to 24 years (aOR, 3.47; 95% CI, 2.26–5.35; $P < 0.001$). Women with concurrent *C. trachomatis* were at a greater risk of testing positive than women who did not have concurrent *C. trachomatis* (aOR, 1.77; 95% CI, 1.05–2.99; $P = 0.033$).

The method of testing used (i.e., culture vs. NAAT), Indigenous status, time in Australia, condomless sex in the last 12 months, sex worker status, and previous STI diagnosis were significantly associated with *T. vaginalis* positivity in the univariable analysis (Table 1) but not in the multivariable analysis (Table 1).

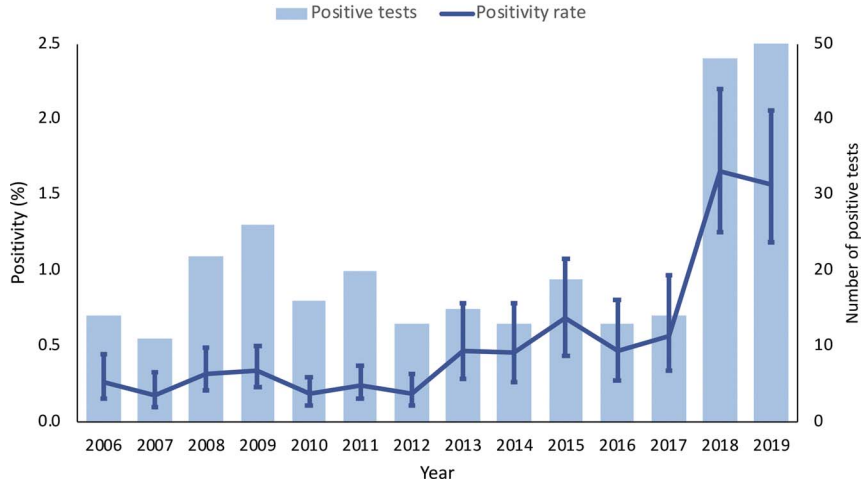

Figure 1. Annual number of cases and test positivity, including the 95% confidence intervals of *T. vaginalis* among women attending the Melbourne Sexual Health Centre between 2006 and 2019. Culture was used as the primary diagnostic method from January 2006 to July 2018. Nucleic acid amplification test was used as the primary diagnostic method between August 2018 and December 2019.

DISCUSSION

Among women attending a sexual health service in Melbourne, Australia, there were 294 *T. vaginalis* cases diagnosed from 2006 to 2019, with an overall positivity of 0.42%. The test positivity increased significantly from 0.27% in 2006 to 1.57% in 2019 ($P_{trend} < 0.001$). There was a statistically significant annual increase of 21% in *T. vaginalis* from 2011 to 2019 in part because of the change in TV diagnostic methods from culture to NAAT. However, the increase in positivity in 2018 when culture was still being used suggests that other factors may have contributed to this increase over time. The sharp increase in test positivity after switching the diagnostic method from culture to NAAT suggests that the low sensitivity of culture might have missed a significant proportion of cases. Concurrent syphilis, injecting drug use in the last 12 months, being 35 years and older, and concurrent chlamydia were all significantly associated with *T. vaginalis* positivity. To our knowledge, this is the largest Australian study on *T. vaginalis* and the only study since 2015.

Our observations suggest that the underlying positivity of *T. vaginalis* in our clinic is increasing and warrants further research into *T. vaginalis*.

These findings are consistent with earlier studies investigating *T. vaginalis* in Australia. A Melbourne-based study found that *T. vaginalis* positivity peaked at 28% in the 1950s before dropping to less than 1% by the early 1990s.[22] The authors hypothesized the decline was due to 2 factors. First is the introduction of nitroimidazoles, which are used to treat *T. vaginalis*. Second, the establishment of the National Cervical Screening Program, which provided free Papanicolaou (Pap) tests to women in Australia. Papanicolaou tests could be assessed for the presence of *T. vaginalis*, and positive findings were included in the final report, essentially acting as a de facto screening program for *T. vaginalis*. A 2011 study reported that 0.2% of 27,602 women attending a sexual health clinic in Sydney tested positive for *T. vaginalis*.[15] The low test positivity in their study is likely due to the use of wet mount for *T. vaginalis* diagnosis, which has a very low sensitivity of 40% to 60% in comparison to culture.[23–27] A 2016 study reported a slightly higher positivity of 0.6% at a public sexual health clinic in Sydney with the use of NAAT (Aptima assay; Hologic Panther system; Hologic).[28] However, this study had a small sample size of 347 women and used urine samples from some clients, which has a lower sensitivity than a vaginal swab.[26] A 2013 study reported a positivity of 0.7% (95% CI, 0.6%–0.8%) in women from urban areas of southeast Queensland.[29] Lusk et al.[30] reported a considerably higher positivity by NAAT (5%) among 356 STI-related symptomatic women in 2 urban Sydney STI clinics in 2010. In comparison, the positivity of *T. vaginalis* in symptomatic women was 0.62% in our study.

The rise in cases coincided with the replacement of culture with NAAT as the primary method of diagnosis. Nucleic acid amplification test has a sensitivity of 95% to 100%,[26,31s,32s] whereas culture has a sensitivity of 63% to 75%.[26,32s] Culture is unable to pick up low parasitic loads,[26] which could have resulted in an underdiagnosis of *T. vaginalis*. A Brazilian study observed a 2-fold increase in cases diagnosed by NAAT when compared with culture.[33s] Kwon et al.[34s] compared wet mount and NAAT on samples collected from women in Sydney. All samples were examined using both methods; the positivity by wet mount was 0.13% (95% CI, 0.03%–0.71%), whereas the positivity by NAAT was 3 times higher (0.38%; 95% CI, 0.14%–0.71%). Consistent with the previous studies, we found that NAAT had a higher positivity (1.68%) compared with culture (0.34%) and suggests that we were missing a substantial number of cases when using culture.

We found that concurrent syphilis was associated with a 22-fold increased risk of *T. vaginalis* positivity in comparison to women who did not have syphilis. A South African study revealed that women at a primary healthcare clinic with a history of syphilis were 1.6 times more likely to test positive for *T. vaginalis*.[35s] A 2021 study by Aung et al.[17] revealed that syphilis cases in women are concentrated in lower socioeconomic suburbs of Melbourne. However, our finding should be interpreted with caution because only a very small number of women had concurrent syphilis (n = 22). Our study highlights the limited information available on *T. vaginalis* coinfections, and more data are needed to better characterize this association.

Similarly, coinfection with *C. trachomatis* was associated with a 2-fold increase in *T. vaginalis* positivity compared with women who did not have *C. trachomatis*. Uddin et al.[15] found that a concurrent STI diagnosis (i.e., *C. trachomatis*, *N. gonorrhoeae*, pelvic inflammatory disease, and early syphilis) was associated with an almost 4-fold increase in the likelihood of a positive *T. vaginalis* test; however, they did not report the risk for individual STIs. There may be several explanations for these associations. Our study is based at an STI clinic, whose clientele are at an inherently higher risk of acquiring STIs compared with the general population. Second, these findings may be due to risky sexual practices in either themselves or their sexual networks. The increased risk of having concurrent *C. trachomatis* or syphilis with *T. vaginalis* suggests that *T. vaginalis* occurs in a core group of women who are at the highest risk of contracting STIs.

Concurrent *N. gonorrhoeae* was not statistically associated in the multivariable analysis with trichomoniasis; however, testing was only recommended for women with symptoms or those who were identified as *N. gonorrhoeae* contacts. The testing policy was changed in August 2017, and *N. gonorrhoeae* testing was then offered to all women attending MSHC, regardless of their presentation.[36s] Several asymptomatic *N. gonorrhoeae* cases were likely missed as a result; a study by Martín-Sánchez et al.[36s] in 2020 found that 48% of women diagnosed with *N. gonorrhoeae* at MSHC were asymptomatic. As a result, it is likely that these women may be at a higher risk of contracting *T. vaginalis*.

We also found that injecting drug use in the last 12 months was strongly associated with *T. vaginalis* positivity. The multivariable analysis showed that these women were 7 times more likely to test positive compared with women who did not report injected drug use in the last 12 months. Our findings are consistent with the 2011 study from Uddin et al.,[15] who found that current injecting drug users were 7 times more likely to test positive for *T. vaginalis* in Sydney. Previous Australian and international studies have demonstrated a relationship between injecting drug use and other STIs.[37s,38s] This could be due to a shared sexual network among injecting drug users with high positivity of STIs. Brookmeyer et al.[39s] investigated sexual risk behaviors among injecting drug users and found that female injecting drug users were more likely to exchange sex for drugs than women who did not inject drugs. Increased outreach to injecting drug users, as well as the promotion of safe sex and asymptomatic screening to this population, could be beneficial in reducing the rates of *T. vaginalis*.

Results indicate that the positivity of *T. vaginalis* is heterogeneous across age groups. Women 35 years and older were 3 times more likely to test positive than women younger than 25 years. Increasing age has been identified as a risk factor in several Australian studies. A 2011 study in Sydney found that the mean age was higher in women who tested positive for *T. vaginalis* (31.5 vs. 28.9 years, $P < 0.001$).[15] Women older than the mean age of 23 years were 3.41 times more likely to test positive among women in rural and remote New South Wales.[16] Interestingly, Bygott and Robson[29] reported that the highest positivity of

T. vaginalis in their study was in girls aged between 10 and 14 years (4%; $P < 0.001$), all of whom were Indigenous. *T. vaginalis* can be asymptomatic for long periods, and some researchers theorize that it can persist in the female genital tract for many years.[40s] Furthermore, the frequency of *T. vaginalis* testing in our clinic and most general practices in Australia is low in comparison to STIs such as *C. trachomatis*. Together, these would allow asymptomatic cases to go undetected and accumulate, resulting in a higher positivity among older women. In addition, the removal of Pap tests from the National Cervical Screening Program may also place upward pressure on *T. vaginalis* rates as they provided opportunistic testing for women, especially those who were asymptomatic. Australian studies have suggested that Pap tests may have contributed to some of the decreases in trichomoniasis locally[15,22]; however, this hypothesis has not been validated in other countries. Our findings highlight the need to recognize the importance of testing for *T. vaginalis* in older women, even if they are asymptomatic.

This study used a very large data set of 69,739 consultations for 14 years, and this enabled us to detect small changes with sufficient power. By collecting the data over an extended period, we were able to look at temporal trends in the data, which has not been done in many studies.

There are several limitations to the study. First, the study population was limited to women who attended a single STI clinic in Melbourne. As the only free public STI clinic in Victoria, it encompasses a wide region; however, women from rural areas are likely to be underrepresented. Therefore, our findings may not be generalizable across the state and the wider Australian population. Second, the clinic population may be biased toward higher-risk women, overestimating the positivity of *T. vaginalis*. In addition, selective testing based on a person's risk profile is also likely to overestimate *T. vaginalis* positivity. However, the generalizability of the point estimates should not influence conclusions drawn from the trends. Third, recall biases may have occurred when reporting sexual practice in the last 12 months and history of STI diagnoses. Fourth, CASI only collected data about current gender and did not collect information about sex at birth. Last, the rise in *T. vaginalis* may be attributable to factors that we were unable to analyze in our study.

The potential reemergence of STIs in women in Melbourne requires interventions aimed at women in high-prevalence groups. These results can be used to provide personalized discussions with clients about risks, including condom use and injecting drug use. Targeted risk education could improve STI rates in general and may improve client understanding of risk reduction. Further testing for *T. vaginalis* among women who are diagnosed with *C. trachomatis* or syphilis may also be warranted.

The rising rates of *T. vaginalis* we have documented are likely to continue without further interventions to mitigate these. Rising rates, particularly in high-prevalence sexual networks with syphilis and injecting drug use, may pose a risk of HIV in these networks given that *T. vaginalis* facilitates HIV transmission.[41s] We propose an update to current screening guidelines to recommend testing in women with the key risk factors associated with *T. vaginalis* at our clinic and within broader community health services.

REFERENCES

1. Rowley J, Vander Hoorn S, Korenromp E, et al. Chlamydia, gonorrhoea, trichomoniasis and syphilis: Global prevalence and incidence estimates, 2016. Bull World Health Organ 2019; 97:548–562P.
2. Gottlieb SL, Low N, Newman LM, et al. Toward global prevention of sexually transmitted infections (STIs): The need for STI vaccines. Vaccine 2014; 32:1527–1535.
3. Petrin D, Delgaty K, Bhatt R, et al. Clinical and microbiological aspects of *Trichomonas vaginalis*. Clin Microbiol Rev 1998; 11:300–317.
4. Carey JC, Yaffe SJ, Catz C. The vaginal infections and prematurity study: An overview. Clin Obstet Gynecol 1993; 36:809–820.
5. Van Gerwen OT, Craig-Kuhn MC, Jones AT, et al. Trichomoniasis and adverse birth outcomes: A systematic review and meta-analysis. BJOG 2021; 128:1907–1915.
6. Hughes JP, Baeten JM, Lingappa JR, et al. Determinants of per-coital-act HIV-1 infectivity among African HIV-1–serodiscordant couples. J Infect Dis 2012; 205:358–365.
7. Mavedzenge SN, Pol BV, Cheng H, et al. Epidemiological synergy of *Trichomonas vaginalis* and HIV in Zimbabwean and South African women. Sex Transm Dis 2010; 37:460–466.
8. McClelland RS, Sangare L, Hassan WM, et al. Infection with *Trichomonas vaginalis* increases the risk of HIV-1 acquisition. J Infect Dis 2007; 195:698–702.
9. Van Der Pol B, Kwok C, Pierre-Louis B, et al. *Trichomonas vaginalis* infection and human immunodeficiency virus acquisition in African women. J Infect Dis 2008; 197:548–554.
10. Yang S, Zhao W, Wang H, et al. *Trichomonas vaginalis* infection-associated risk of cervical cancer: A meta-analysis. Eur J Obstet Gynecol Reprod Biol 2018; 228:166–173.
11. Guy R, Ward J, Wand H, et al. Coinfection with *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis*: A cross-sectional analysis of positivity and risk factors in remote Australian aboriginal communities. Sex Transm Infect 2015; 91:201–206.
12. Brown EEJ. STI/HIV structural and socio structural barriers among Black women residing: In the rural southwest. J Multicult Nurs Health 2003; 9:40–48.
13. Denison HJ, Bromhead C, Grainger R, et al. Barriers to sexually transmitted infection testing in New Zealand: A qualitative study. Aust N Z J Public Health 2017; 41:432–437.
14. Wong J, Chan KK, Boi-Doku R, et al. Risk discourse and sexual stigma: Barriers to STI testing, treatment and care among young heterosexual women in disadvantaged neighbourhoods in Toronto. Can J Hum Sex 2012; 21:75.
15. Uddin RN, Ryder N, McNulty AM, et al. *Trichomonas vaginalis* infection among women in a low prevalence setting. Sex Health 2011; 8: 65–68.
16. Ryder N, Woods H, McKay K, et al. *Trichomonas vaginalis* prevalence increases with remoteness in rural and remote New South Wales, Australia. Sex Transm Dis 2012; 39:938–941.
17. Aung ET, Chen MY, Fairley CK, et al. Spatial and temporal epidemiology of infectious syphilis in Victoria, Australia, 2015–2018. Sex Transm Dis 2021; 48:e178–e182.
18. Jasek E, Chow EP, Ong JJ, et al. Sexually Transmitted Infections in Melbourne, Australia from 1918 to 2016: Nearly a century of data. Commun Dis Intell Q Rep 2017; 41:E212–E222. Available at: http://europepmc.org/abstract/MED/29720070.
19. Australian Bureau of Statistics. Australian Statistical Geography Standard (ASGS): Volume 5—Remoteness Structure, July 2016. Cat. No. 1270.0. 55.005. Canberra, Australia: Australian Bureau of Statistics, 2018.
20. Fagan T. Exact 95% confidence intervals for differences in binomial proportions. Comput Biol Med 1999; 29:83–87.
21. Victoria State Department of Health. Syphilis Cases Continue to Rise in Victoria. Victoria, Australia: Victoria State Department of Health, 2019. Available at: https://www2.health.vic.gov.au/about/news-and-events/healthalerts/rising-syphilis-cases-august-2018. Accessed October 12, 2021.
22. Marrone J, Fairley CK, Saville M, et al. Temporal associations with declining *Trichomonas vaginalis* diagnosis rates among women in the state of Victoria, Australia, 1947 to 2005. Sex Transm Dis 2008; 35: 572–576.
23. Wiese W, Patel SR, Patel SC, et al. A meta-analysis of the Papanicolaou smear and wet mount for the diagnosis of vaginal trichomoniasis. Am J Med 2000; 108:301–308.
24. Van Der Pol B. Clinical and laboratory testing for *Trichomonas vaginalis* infection. J Clin Microbiol 2016; 54:7–12.
25. Adjei C, Boateng R, Dompreh A, et al. Prevalence and the evaluation of culture, wet mount, and ELISA methods for the diagnosis of *Trichomonas vaginalis* infection among Ghanaian women using urine and vaginal specimens. Trop Med Health 2019; 47:33.

26. Nye MB, Schwebke JR, Body BA. Comparison of APTIMA *Trichomonas vaginalis* transcription-mediated amplification to wet mount microscopy, culture, and polymerase chain reaction for diagnosis of trichomoniasis in men and women. Am J Obstet Gynecol 2009; 200:188.e1–188.e7.
27. Knox J, Tabrizi SN, Miller P, et al. Evaluation of self-collected samples in contrast to practitioner-collected samples for detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* by polymerase chain reaction among women living in remote areas. Sex Transm Dis 2002; 29:647–654.
28. Tilley DM, Dubedat SM, Lowe P, et al. Genital *Trichomonas vaginalis* is rare among female attendees at a Sydney metropolitan sexual health clinic. Aust N Z J Public Health 2016; 40:95–96.
29. Bygott JM, Robson JM. The rarity of *Trichomonas vaginalis* in urban Australia. Sex Transm Infect 2013; 89:509–513.
30. Lusk MJ, Naing Z, Rayner B, et al. *Trichomonas vaginalis*: Underdiagnosis in urban Australia could facilitate re-emergence. Sex Transm Infect 2010; 86:227–230.

For further references, please see "Supplemental References," http://links.lww.com/OLQ/A849.